Thallium in Hair by ICP/MS

1 Introduction

Thallium is a toxic metal that may cause gastrointestinal, neurological or dermatological symptoms. Thallium can be detected in hair following chronic exposure or following survival of an acute exposure. This procedure is used to detect and quantitate thallium (Tl) in hair specimens.

2 Scope

This procedure is used to selectively assay thallium in hair specimens by Inductively Coupled Plasma Mass Spectrometry (ICP/MS) with a collision cell. Hair samples are first chemically digested then diluted, spiked with an internal standard and analyzed.

3 Principle

Hair specimens are weighed, completely digested in Tetramethylammonium hydroxide (TMAH), mixed with the Iridium Internal Standard Working Solution, diluted up to 10 mL in Dilute Nitric Acid in Water Solution and analyzed contemporaneously with a complete matrix matched calibration curve, a Negative Control Hair sample and duplicate sets of Positive Control Hair samples.

4 Specimens

This procedure is validated for hair. Two 5.5 mg samples are analyzed. However, if it is suspected that the thallium concentration is above the procedure's linear range, smaller sample sizes or further dilutions of the samples may be analyzed.

5 Equipment/Materials/Reagents

a. 10 mL and 25 mL volumetric flasks, class A, PMP (VITLAB® or equivalent)

b. 5 mL 12 x 75 mm round bottom tubes with caps, PP (Falcon® or equivalent)

c. 15 mL and 50 mL conical tubes with screw tops, PP (Falcon® or equivalent)

d. 100 mL volumetric flask, class B, PP (Nalgene® or equivalent)

e. 1 L volumetric flask, class B, PP (Nalgene® or equivalent)

f. 25 mL plastic graduated cylinder (Nalgene® or equivalent)

g. Concentrated nitric acid (Optima grade)

h. Water (Deionized, 18 MΩ)

i. Dilute Nitric Acid in Water Solution (v:v):
To a 1 L Nalgene® volumetric flask, add approximately 800 mL of deionized water. Add 40 mL of Optima grade concentrated nitric acid, fill to the mark with deionized water and mix well. Store at room temperature in plastic. Stable for at least one year.

j. Tetramethylammonium hydroxide (TMAH), 25% w/w aqueous solution (Electronic grade)

k. Methanol (HPLC grade or better)

l. Balance capable of measuring ± 0.1 mg

m. Routine laboratory supplies including but not limited to: pipettes, disposable pipettes, forceps, hand shears, etc.

n. Vortexer

o. Inductively Coupled Plasma-Mass Spectrometer with a collision cell installed (Thermo-Fisher iCAP Q or equivalent)

p. Autosampler (CETAC ASX-260 or equivalent)

q. Cryogrinder (optional)

r. Acetone (Reagent grade or better)

s. Heating block

6 Standards and Controls

a. Iridium Internal Standard Stock Solution (1 µg/mL):
Purchased from SPEX CertiPrep or an equivalent supplier. Stability and storage determined by manufacturer.

b. Iridium Internal Standard Working Solution (10 µg/L):
To a 100 mL Nalgene® volumetric flask that has been rinsed with Dilute Nitric Acid in

Water Solution, add about 80 mL of Dilute Nitric Acid in Water Solution. Add 1 mL of Iridium Internal Standard Stock Solution, fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

c. Thallium Calibrator Stock Solution (1000 mg/L in Dilute Nitric Acid in Water Solution): Purchased from SPEX CertiPrep or an equivalent supplier. Stability and storage determined by manufacturer.

d. Thallium Calibrator Working Stock Solution - High (5 mg/L in Dilute Nitric Acid in Water Solution):
To a 25 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 15 mL of Dilute Nitric Acid in Water Solution. Add 0.125 mL of the Thallium Calibrator Stock Solution (1000 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

e. Thallium Calibrator Working Stock Solution - Low (0.5 mg/L in Dilute Nitric Acid in Water Solution):
To a 10 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 7 mL of Dilute Nitric Acid in Water Solution. Add 1.0 mL of the Thallium Calibrator Working Stock Solution High (5 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

f. Thallium Calibrator Working Solutions (1 µg/L – 250 µg/L):
The following table shows the preparation of the individual Calibrator Working Solutions. The Calibrator Working Solutions are prepared in individual 25 mL class A volumetric flasks that have been washed with Dilute Nitric Acid in Water Solution. Store at room temperature in plastic. Fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Stable for at least one year.

Calibrator Working Solutions (µg/L) (Prepared in 25-mL volumetric flasks)	Volume of Thallium Calibrator Working Stock Solution - High (5 mg/L) (µL)	Volume of Thallium Calibrator Working Stock Solution - Low (0.5 mg/L) (µL)	Corresponds to xx ng Thallium per mg of hair as prepared
1		50	0.02
2.5		125	0.05
5		250	0.10
10	50		0.20
25	125		0.50
50	250		1.00
75	375		1.50
100	500		2.00
175	875		3.50
250	1250		5.00

g. Thallium Control Stock Solution (10,000 mg/L in Dilute Nitric acid solution)
Purchased from High Purity Standards or an equivalent supplier. Stability and storage determined by manufacturer.

h. Thallium Intermediate Control Solution High (10 mg/L in Dilute Nitric Acid in Water Solution):
To a 25 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 15 mL of Dilute Nitric Acid in Water Solution. Add 0.025 mL of the Thallium Control Stock Solution (10,000 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

i. Thallium Intermediate Control Solution Low (0.5 mg/L in Dilute Nitric Acid in Water Solution):
To a 10 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 15 mL of Dilute Nitric Acid in Water Solution. Add 0.5 mL of the Thallium Intermediate Control Solution High (10 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

j. Thallium High Control Working Solution (200 µg/L in Dilute Nitric Acid in Water Solution):
To a 25 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 15 mL of Dilute Nitric Acid in Water Solution. Add 0.5 mL of the Thallium Intermediate Control Solution High (10 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

k. Thallium Low Control Working Solution (7.5 µg/L in Dilute Nitric Acid in Water Solution):
To a 25 mL class A volumetric flask that has been rinsed with Dilute Nitric Acid in Water Solution, add about 15 mL of Dilute Nitric Acid in Water Solution. Add 0.375 mL of the Thallium Intermediate Control Solution Low (0.5 mg/L), fill to the mark with Dilute Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

l. Negative Control Hair:
Prepared from in-house anonymous donations. Collected negative specimens are screened for thallium. If thallium is present, the level must be below 0.02 ng/mg (1 µg/L). Store at room temperature in paper. Stable indefinitely.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

1. Prepare negative control hair digest for the calibration curve and controls:

    a. To a labeled 15 mL Falcon® tube:
        1. Add a minimum of 100 mg of negative hair. Accurately record the mass to the nearest 0.1 mg. Alternatively, a larger hair sample may be cryoground to mix it well. (The negative control hair digest will be prepared in the same manner as the unknown sample.)
        2. Based upon the recorded weight, add enough TMAH to establish a solution of 5 mg of hair per 100 µL of TMAH. (For example, 2000 µL TMAH is needed for 100 mg of hair.)
    b. Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)

2. Prepare the calibration curve:

    a. To individually labeled 10 mL Vitlab® volumetric flasks that have been washed with Dilute Nitric Acid in Water Solution add:
        1. Approximately 5 mL Dilute Nitric Acid in Water Solution;
        2. 100 µL of negative hair digest (prepared in 8.1 above);
        3. 100 µL of each Calibrator Working Solutions (prepared in 6.f above);
        4. 100 µL of the Iridium Internal Standard Working Solution (prepared in 6.b above);
        5. Dilute Nitric Acid in Water Solution to the mark, mix well;
    b. Transfer controls to labeled 15 mL Falcon® tubes.
    c. Prepare a zero calibrator and a Negative Control in the same manner but add 100 µL of deionized water instead of a Calibrator Working Solution. Additionally, prepare a Blank (no matrix added) with Internal Standard by adding 100 µL Iridium Internal Standard Working Solution to 10 mL with Dilute Nitric Acid in Water Solution.
    d. Prepare Negative Control in duplicate.

3. Have a second chemist prepare the Positive Controls:

    a. To individual 10 mL Vitlab® volumetric flasks that have been washed with Dilute

Nitric Acid in Water Solution add:

1. Approximately 5 mL Dilute Nitric Acid in Water Solution;
2. 100 µL of negative hair digest (prepared in 8.1 above);
3. 100 µL of the appropriate Control Solution (7.5 or 200 µg/L);
4. 100 µL of the Iridium Internal Standard Working Solution;
5. Dilute Nitric Acid in Water Solution to the mark, mix well;

b. Transfer controls to labeled 15 mL Falcon® tubes.
c. Prepare positive controls in duplicate.

Note: As prepared, the Low Control corresponds to 0.15 ng thallium / mg hair and the High Control corresponds to 4 ng thallium / mg hair.

4. Prepare the unknown samples:

a. Optional: If specimen size allows, positive specimens are repeated following an external wash. Submerge the hair specimen in acetone, and mix for 10 minutes. Repeat the process three times with deionized water for ten minutes each, and finally for 10 more minutes with acetone. When the unknown hair specimen is washed in this manner, a negative control should also be contemporaneously washed and analyzed alongside the unknown. Allow the specimen to dry in a fume hood or in a heating block at 50°C.
b. To individually labeled 5 mL (12 x 75) Falcon® tubes:
    1. Add a minimum of 5.5 mg of specimen hair in duplicate. Accurately record the mass to the nearest 0.1 mg.
       Note: 5.5 mg is a small amount of hair. In order to ensure that a representative hair sample is analyzed, a larger amount of hair may be cut into small snippets and mixed before removing the 5.5 mg sample. Alternatively, a larger hair sample may be cryoground to mix it well. (The negative control hair digest will be prepared in the same manner as the unknown sample.)
    2. Based upon the recorded weight, add enough TMAH to establish a solution of 5 mg of hair per 100 µL of TMAH. (110 µL TMAH is added to 5.5 mg hair.)
c. Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)
d. To individual 10 mL Vitlab® volumetric flasks that have been washed with Dilute Nitric Acid in Water Solution add:
    1. Approximately 5 mL Dilute Nitric Acid in Water Solution;
    2. 100 µL of unknown hair digest (prepared above);
    3. 100 µL of water;
    4. 100 µL of the Iridium Internal Standard Working Solution;
    5. Dilute Nitric Acid in Water Solution to the mark, mix well;
e. Transfer to labeled 15 mL Falcon® tubes.

5. Centrifuge all samples at 3500 rpm for 15 minutes and transfer to new labeled 15 mL Falcon® tubes before analysis by ICP/MS.

6. When setting up the sequence, run a nitric acid blank four times followed by a Blank with Internal Standard, followed by a Negative Control. Then run the calibrators, followed by the unknowns bracketed by the Positive Controls. A Blank with Internal Standard followed by a Negative Control may be analyzed between specimens and before the Positive Controls.

9 Instrumental Conditions

The following conditions are written to follow Thermo-Fisher's LabBooks software package.

Analytes: Tl (Thallium) and Ir (Iridium)

Acquisition parameters:

Identifier	Dwell time (s)	Channels	Spacing (u)	Measurement mode	Resolution
205Tl (KED)	0.05	1	0.1	KED	Normal
191Ir (KED)	0.05	1	0.1	KED	Normal
				# sweeps = 20	

Monitor analytes:

	Uptake (s)	Wash (s)
Minimum	30	30
Maximum	300	300

Survey scan settings:

Start mass (u)	End mass (u)	Dwell Time (s)	Spacing (u)	Resolution	Measurement mode
4.60	245.00	0.01	0.2	Normal	KED

Interference correction: not applicable

Standards: covered in cal/ctrl section

Quantification:

Analyte	Measurement mode	Quantify	Internal Standard	Fit type	Weighting	Forcing	Use for Semi-Quant
205Tl (KED)	KED	Yes	193 Ir (KED)	Linear	None	Blank	Yes
191Ir (KED)	KED	No	Use as Internal Standard	Linear	None	Blank	Yes

IS Recovery		Low warning limit: 80%			Low failure limit: 75%		
		High warning limit: 120%			High failure limit: 125%		

Ratios: not applicable

Quality Control:

Calibration Tests / Laboratory Control Standard (LCS)

Analyte	Low failure limit	Low warning limit	High warning limit	High failure limit
205Tl (KED)	75%	80%	120%	125%

Continuous Tests / Relative Stability Verification (RSV)

Analyte	Verify	Ignore concentration below	unit	Concentration warning limit	Concentration failure limit
205Tl (KED)	concentration	10	ppb	5%	10%

Autosampler:

Time Settings:	Wash Time (s):	200	Take up Time (s):	45
Rack Settings:	Rack 1 Type:	60-vials (12x5)	Rack 2 Type:	60-vials (12x5)
Autotune Settings:	Autotune rack:	Standard	Autotune vial:	1
Rinse settings	Rinse Rack:	Rinse Station		

10 Decision Criteria

Normal hair contains thallium levels less than 0.02 ng/mg. Chronic thallium hair levels in occupational workers have ranged from 0.020 to 0.57 ng/mg. During acute thallium toxicity, thallium hair levels can be greater than 1 ng/mg.

10.1 Batch Acceptance Criteria

Thallium should not be detected in the Negative Control Hair specimen above a level of 0.02 ng/mg.

Thallium in the Positive Control Hair specimens should quantitate within ±20% of the target value.

See the Guidelines for Toxicological Quantitations standard operating procedure (Tox 101) for more information.

10.2 Sample Acceptance Criteria

The Iridium (Internal Standard) response in the unknown specimen(s) should fall within 80% and 120% of the Internal Standard response for the initial blank in the run.

11 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the intensity for each calibrator level and the internal standard. The curve is forced through a Negative Control / Zero Calibrator specimen with no weighting. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

For hair specimens, 5 mg of hair is used in the place of 0.1 mL of liquid. Therefore, results as received from the instrument for hair should be divided by 50. For example, if a result of 100 µg/L of Tl is obtained for a hair specimen, that corresponds to 2 ng Tl per mg of hair.

When a hair specimen contains thallium above the method's lower limit of quantitation, it may be analyzed again using a method of standard addition to verify the Tl concentration.

12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

13 Limitations

a. Limit of Detection = 0.02 ng/mg (1 µg/L)

b. Limit of Quantitation = 0.02 ng/mg (1 µg/L)

c.

Hair:	@ 0.15 ng/mg (7.5 µg/L)	@ 2.4 ng/mg (1200 µg/L)	@ 4 ng/mg (200 µg/L)
% Bias	-3.63%	-3.68%	-2.91%
% Repeatability	1.83%	1.63%	1.16%
% Intermediate Precision	2.49%	2.79%	2.63%

d. Interferences: no endogenous material/matrices interfered with the analysis of thallium. Additionally, elemental standards of gallium, arsenic, indium, rhodium, cadmium, lead, mercury, bismuth, holmium, lithium, scandium, terbium, yttrium, barium, cerium, cobalt and uranium did not interfere with the thallium signal.

e. Carryover: No carryover was observed when a negative control hair specimen was analyzed immediately following a 5 ng/mg (250 ug/L) calibrator.

f. Processed sample stability: Sample stability was evaluated up to four days after the initial analysis and remained within ± 20% with refrigerated storage.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Guidelines for Toxicological Quantitations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

FBI Laboratory Safety Manual.

Kempson I.M., et al. (2012) A Comparison of Washing Methods for Hair Mineral Analysis: Internal Versus External Effects. Biol Trace Elem Res. 150 (1-3).

Hirata, et al. (1998) A Probable Case of Chronic Occupational Thallium Poisoning in a Glass Factory. Industrial Health. 36, 300-303.

Rusniak (2002) Thallium and Arsenic Poisoning in a Small Midwestern Town. Annals of Emergency Medicine 39(3):307-311

Rev. #	Issue Date	History
0	06/03/2016	New document.

Approval

Redacted - Signatures on File

Appendix 1: Abbreviated version of the Procedure for bench use.

Redacted - Form on File

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